

WHAT IS CLAIMED IS:

- 00305530-001499
1. A recombinant RNA molecule comprising a binding site specific for an RNA-directed RNA polymerase of a negative strand RNA virus, operatively linked to a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation.
2. The recombinant RNA molecule of Claim 1 in which the polymerase binding site comprises the polymerase binding site contained in the 3'-noncoding flanking sequence of an influenza genome vRNA segment.
3. The recombinant RNA molecule of Claim 1 in which the polymerase binding site comprises the terminal 12 nucleotides of the 3'-terminus of an influenza genomic segment.
4. The recombinant RNA molecule of Claim 1 in which the 3'-noncoding viral sense flanking sequence of influenza comprises the following sequence:
5'-CCUGCUUU^CGCU-3'
5. A recombinant RNA molecule comprising a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to a 3'-noncoding flanking sequence of an influenza vRNA containing the viral polymerase binding site, and to a 5'-noncoding flanking sequence of an influenza vRNA.
6. The recombinant RNA molecule of Claim 5 in which the 5'-noncoding flanking sequence of an

influenza vRNA comprises the first 22 nucleotides of the 5'-terminus of an influenza genomic segment.

7. The recombinant RNA molecule of Claim 5 in which the 5'-noncoding flanking sequence of an influenza vRNA comprises the following sequence:
- 5'-AGUAGAAACAAGGGUGUUUUU-3'.

8. A recombinant RNP comprising the recombinant RNA molecule of Claim 1 complexed with the purified RNA-directed RNA polymerase.

9. A recombinant RNP comprising the recombinant RNA molecule of Claim 2 complexed with a purified influenza viral polymerase.

10. The recombinant RNP of Claim 9 in which the influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, in which the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0 M CsCl.

11. A recombinant RNP comprising the recombinant RNA molecule of Claim 5 complexed with a purified influenza viral polymerase.

12. The recombinant RNP of Claim 11 in which the influenza viral polymerase is obtained from RNPs fractionated by centrifugation on a CsCl gradient, in which the purified influenza viral polymerase is isolated from the region of the gradient correlating to 1.5 to 2.0 M CsCl.

13. A chimeric virus comprising influenza virus containing a heterologous RNA sequence comprising the

reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to an influenza viral polymerase binding site.

- 5 14. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 1 of influenza virus.
- 10 15. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 2 of influenza virus.
- 15 16. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 3 of influenza virus.
- 20 17. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 4 of influenza virus.
- 25 18. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 5 of influenza virus.
- 30 19. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 6 of influenza virus.
- 35 20. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 7 of influenza virus.
21. The chimeric virus of Claim 13 in which the heterologous RNA sequence is contained within segment 8 of influenza virus.

22. A chimeric virus comprising influenza virus containing in addition to its eight genomic segments an additional RNA segment containing a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to an influenza viral polymerase binding site.

23. The chimeric virus of Claim 22 further containing a selectable coding sequence such that the bicistronic mRNA coding sequence is stably expressed.

24. A chimeric virus comprising a negative strand RNA virus containing a heterologous RNA sequence comprising the reverse complement of a bicistronic mRNA coding sequence containing an internal sequence that mediates internal initiation of translation, operatively linked to a polymerase binding site of the negative-strand RNA virus.

25. The chimeric virus of Claim 24 further containing a selectable coding sequence such that the bicistronic mRNA coding sequence is stably expressed.

26. A recombinant DNA molecule encoding the recombinant RNA molecule of Claim 1 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

27. A recombinant DNA molecule encoding the recombinant RNA molecule of Claim 2 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

28. A recombinant DNA molecule encoding the recombinant RNA molecule of Claim 5 operatively linked to a transcription control element that binds a DNA-directed RNA polymerase.

5 29. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of Claim 8 so that the heterologous gene is expressed in the culture.

10 30. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of Claim 9 so that the heterologous gene is expressed in the culture.

15 31. A method for gene expression, comprising culturing a host cell transfected with the recombinant RNP of Claim 11 so that the heterologous gene is expressed in the culture.

20 32. A method for producing a chimeric negative-strand RNA virus, comprising culturing a host cell transfected with the recombinant RNP of Claim 8 and infected with a parental strain of the negative strand RNA virus, and recovering the chimeric virus
25 from the culture.

33. A method for producing a chimeric influenza virus, comprising culturing a host cell transfected with the recombinant RNP of Claim 9 and infected with a parental strain of influenza, and
30 recovering the chimeric influenza virus from the culture.

34. A method for producing a chimeric
35 influenza virus, comprising culturing a host cell

transfected with the recombinant RNP of Claim 11 and
infected with a parental strain of influenza, and
recovering the chimeric influenza from the culture.

add B1

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add D1

add F2

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